Invasive Plant Science and Management 2010 3:26–31

White Sweetclover (*Melilotus albus*) and Narrowleaf Hawksbeard (*Crepis tectorum*) Seed Germination after Passing through Moose

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White sweetclover and narrowleaf hawksbeard are nonindigenous invasive plant species in Alaska that are rapidly spreading, including into areas that are otherwise free of nonindigenous plants. There has been concern that native moose could be dispersing germinable seed from these plants after ingestion. To address this concern, a study was conducted involving tame moose at the University of Alaska Fairbanks Agriculture and Forestry Experiment Station, Matanuska Experiment Farm, Palmer, AK. Objectives were to determine if seeds from these two plant species could survive mastication and digestive passage through moose, whether this passage impacted seed germination, and whether seed passage rates were the same as similar sized Cr-mordanted fiber. In this study, narrowleaf hawksbeard seed rarely survived mastication and digestion with only five seedlings recovered from 42,000 germinable seed fed to the moose. About 16% of germinable white sweetclover seed (3,595 of 22,000) fed to the moose produced seedlings. Most of the sweetclover seedlings came from feces produced 2 and 3 d after feeding. In two moose, sweetclover seedlings were grown from fecal material that was passed 11 d after feeding, raising the possibility that seeds could be transported long distances after ingestion. Cr-mordanted fiber passage did not closely follow seedling producing seed, possibly because time in the rumen might reduce seed germination. Once roadsides in Alaska become infested with white sweetclover, moose can then serve as a transport vector of these weeds into river channels and floodplains, which are distant from roads. This information will impact white sweetclover management programs and alert land managers to the potential for other instances of wildlife-mediated seed dispersal.

Nomenclature: Narrowleaf hawksbeard, *Crepis tectorum* L.; white sweetclover, *Melilotus albus* Desr.; moose, *Alces alces* L.

Key words: Endozoic distribution, endozoochorous dispersal, seed transport.

White sweetclover (*Melilotus albus* Desr.) and narrowleaf hawksbeard (*Crepis tectorum* L.) are nonindigenous invasive plant species in Alaska that are expanding into disturbed areas and from there into areas previously free of nonindigenous plants (Figure 1). White sweetclover is a nitrogen-fixing forage crop that is native to Europe and western Asia. An annual white sweetclover variety was imported to Alaska in 1913 as a potential forage crop, but had poor survivability (Irwin 1945). After a number of generations, escaped white sweetclover populations in Alaska have been selected for a biennial habit (Klebesadel 1994), more responsiveness to shortening photoperiods, and increased rates of freezing tolerance response to lowering temperatures (Klebesadel 1993). White sweetclover infestations now occur along many roadsides, in urban areas, and along sections of several river flood plains (Conn et al. 2008). Narrowleaf hawksbeard is native to Europe, and is a more recent introduction in Alaska, identified in Fairbanks before 1968 (Hulten 1968). It is a rapidly-maturing annual that produces wind-blown seeds throughout the summer and autumn, and is invading

DOI: 10.1614/IPSM-09-034.1

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Interpretive Summary

White sweetclover and narrowleaf hawksbeard are two widespread, nonindigenous plant species that are rapidly expanding in Alaska. White sweetclover is expanding most rapidly along roadsides, river flood plains, and in disturbed urban areas. Narrowleaf hawksbeard, with a wind-blown seed, has been found in recently burned natural areas. There was concern that moose could be a vector for the spread of these two species after ingesting germinable seed. A study was conducted to determine whether and for how long germinable seed could be found in moose feces after ingestion. The results of this study indicate narrowleaf hawksbeard is not a species of concern for moose-mediated dispersal because only five seedlings were produced after the ingestion of over 42,000 germinable seed. However 16% of germinable white sweetclover seed survived mastication and digestion in moose, and seedling-producing seed were found 11 d after feeding. Management programs will need to be developed to limit the availability of white sweetclover seed for moose. Management could include techniques such as mowing after flowering so that any seed is covered in snow during the winter or use of herbicides to prevent seed production. Scouting for new infestations of white sweetclover spreading from established populations will need to factor in moose trails and the distance moose can move over several days, which can be several km in the home range or several tens of km if the moose is migrating.

disturbed ground and agricultural lands throughout Alaska (AKEPIC Database 2008).

Passage of seeds through animal digestive systems can result in enhanced, reduced, or neutral impacts on subsequent germination (for review see Traveset 1998). In the case of nonindigenous invasive plant species, successful passage of seed represents the potential for herbivores to serve as transport vectors, thus broadening and enhancing seed dispersal. Foraging animals can, therefore, facilitate establishment of new invasive plant populations quite far from the original infestation. Passage of spotted knapweed (*Centaurea stoebe* L.), an important nonindigenous invasive species in western North America, through mule deer (*Odocoileus hemionus*) was confirmed with 8% viability in 11% of the original seed found in the fecal material, representing a reduction of 5,000 to 43 seed (Wallander et al. 1995). This survival of spotted knapweed seed after mastication and digestion confirmed that mule deer can mediate dispersal of spotted knapweed, especially given that viable seed were being passed 10 d postfeeding (Wallander et al. 1995). Increased seed germination after passage through animal digestive systems is an example of mutualism and has been documented for such combinations as black nightshade (*Solanum nigrum* L.) and two bird species [European blackbird (*Turdus merula* L.) and European starling (*Sturnus vulgaris* L.)] (Clergeau 1992) and purple mombin (*Spondias purpurea* L.) and a deer species (*Odocoileus virginianus* Zimmermann) (Mandujano et al. 1994).

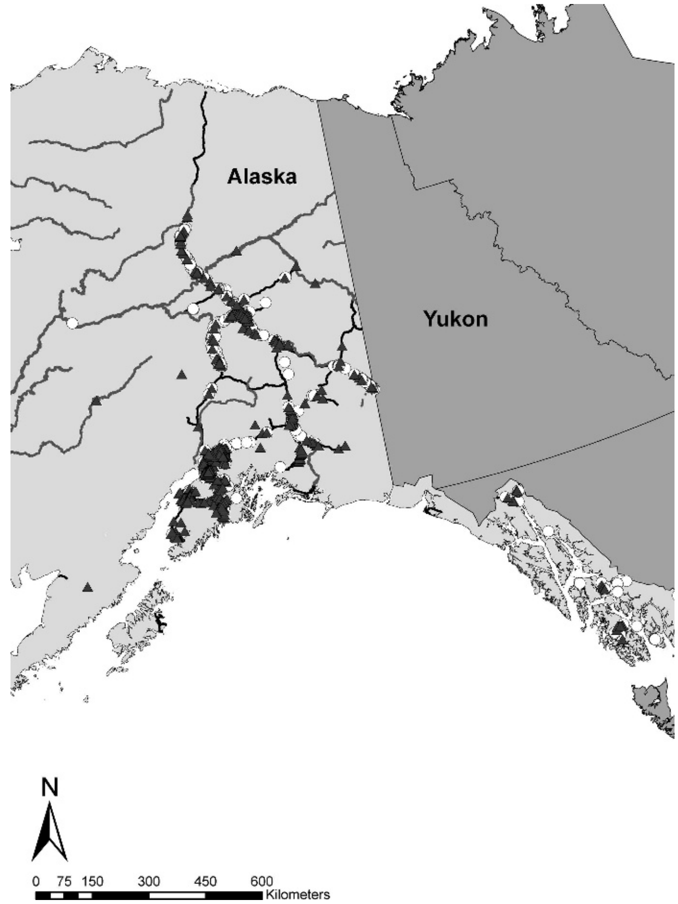

Figure 1. White sweetclover (circles) and narrowleaf hawksbeard (triangles) infestations in Alaska (produced with data from AKEPIC Database 2008). Roads are solid black lines and major rivers are solid grey lines.

There has been recent interest in the relationship between ingested particle size and passage time in ungulate animals (Schwarm et al. 2008, 2009). Because both white sweetclover and narrowleaf hawksbeard seed are similar in size, a comparison with a similar-sized mordanted fiber would provide useful insights into factors potentially influencing seed passage time.

We have observed moose (*Alces alces* L.) utilizing white sweetclover in Alaska as a minor component of their diet in the Yukon province of northern Canada from late summer into winter when seeds on 2-yr-old plants are mature (Bruce Bennet, personal communication). To date there has been no research documenting moose use of narrowleaf hawksbeard; however, narrowleaf hawksbeard has been observed growing in feces deposited by wild moose in burned areas in interior Alaska (Katie Villano, personal communication). If the seed of either species are ingested by moose and survive passage through the gut in germinable condition, this could represent a significant pathway for spread of these nonindigenous invasive plant species.

The objectives of this research were to determine: (1) whether white sweetclover and narrowleaf hawksbeard seed could survive mastication and digestive passage through moose; (2) if this passage enhanced, reduced, or had no impact on the germination of any surviving seeds; and (3) whether a similar-sized material passed through moose at similar rates.

Materials and Methods

Seed Collection. White sweetclover seed were collected from roadsides near Fairbanks, AK, in August and September 2008. Narrowleaf hawksbeard seed were collected throughout the summer of 2008 around the University of Alaska Fairbanks campus. Seed were stored at 4 C. To control the experiment, the germination rate of untreated sweetclover and hawksbeard seed were evaluated. Seed germination (measured as the proportion of germinated seeds) of white sweetclover was 34 $\pm$ 3% (standard deviation) and narrowleaf hawksbeard was 93 $\pm$ 9%, when germinated in a Petri dish on filter paper in a seed germinator with a 12/12 day/night length at 15 C (Najda et al. 1982). Germination was retested on white sweetclover after a 1 hr soak in sulfuric acid (98%) and a triple-rinse with distilled water to break dormancy before germinating as above; however, germination remained low at 39 $\pm$ 6%.

Feeding and Feces Collection. Four tame, adult female moose (5 to 8 years old), with body mass ranging from 341 to 370 kg, housed at the University of Alaska Fairbanks, Agriculture and Forestry Experiment Station, Matanuska Experiment Farm in Palmer, AK, were used in the study. These animals were well accustomed to experimental protocol used in this experiment, and readily utilized the facilities under which they were confined during the experiment. Experiments and handling procedures for animals were approved by the Institutional Animal Care and Use Committee, University of Alaska Fairbanks under protocol #07-21.

Based on three 100-weight counts for both plant species, total seed numbers were determined. White sweetclover was divided into four lots of 15,200 seed (approximately 5,500 germinable seed) and narrowleaf hawksbeard was divided into four lots of 11,400 seed (approximately 10,600 germinable seed). On October 19, 2008, each dose of seeds was mixed with approximately 100 ml of canned pumpkin immediately before feeding. The pumpkin ensured that no seeds were dropped during ingestion.

Moose were fed a diet of brome grass (*Bromus inermis* Leyss.) silage beginning 21 d before and throughout the feeding trial (Figure 2). A subsample of the silage was cleaned of large straw and the remainder of the material

Figure 2. Dr. William Collins feeding white sweetclover and narrowleaf hawksbeard mixed with 100 ml of canned pumpkin to moose and the University of Alaska Fairbanks Agriculture and Forestry Experiment Station, Matanuska Experiment Farm, Palmer, AK.

was placed in a 28 by 53 by 6 cm plastic tray in a greenhouse and watered to determine whether the silage contained germinable seed. Mean digestibility of the silage to the moose was 0.653 $\pm$ 0.0190.

Moose were kept in separate digestion-balance stalls and all fecal material was collected, bagged, and frozen at -10 C each day for the next 11 d.

Seedling Growth. On November 17, 2008 all fecal material was removed from the freezer and placed in a greenhouse (18 C) to thaw. The following day, fecal material was placed in 28 by 53 by 6 cm plastic trays to a depth of 2 to 4 cm, without disrupting the natural shape of the fecal pellets. Depending on volume, three to seven trays were needed for each moose for each day. The greenhouse was kept at 18 C with a 13/11 hr day/night cycle. Trays were watered every 4 to 5 d, as needed, and covered with plastic domes to reduce evaporation. Before watering, identifiable white sweetclover and narrowleaf hawksbeard seedlings were counted and removed. Seedlings from these species were mainly forming from seed located on the surface of the fecal pellets rather than from the pellet interior. Unknown seedlings that were not white sweetclover or narrowleaf hawksbeard were transplanted and grown until identified. Numbers for each of the other plant species were not recorded.

After 7 wk, when new seedlings were rare, fecal material was air-dried in the greenhouse for 2 wk, weighed, and combined. In order to determine the amount of germinable seed inside the pellets, which, over the course of the study had developed a hard surface, four subsamples from the combined fecal material (> 1 kg each) were sent to the USDA–ARS–Subarctic Agricultural Research Unit in Fairbanks, AK for additional processing and analysis. Half of each of these subsamples was weighed, dried at 60 C for 48 hr and reweighed to determine percent dry weight. The other half of each sample was sieved through a 5.6 mm mesh (No. 3.5) after moistening to achieve a soft consistency. The sieved fecal material was placed in 28 by 53 by 6 cm plastic trays to a depth of 2 cm and watered. These trays were placed in a seed germinator set to 15 C with a 12/12 hr day/night cycle. Seedlings were counted and, based on dry weight calculations and seedling number for each moose and day of passage in the greenhouse, seedling numbers were adjusted to reflect the additional seedlings found inside the sample pellets. ANOVA (SAS 1988) was used to detect differences ($P \leq 0.05$) among moose and days after feeding. Mean separations of significant effects were evaluated with Fisher's Protected LSD test ($P \leq 0.05$).

Passage of Cr Marked Fiber. At the same time moose were fed the seeds, 40 g of chromium (Cr)-mordanted fiber (Uden et al. 1980) was inserted directly into the rumens of two of the moose, which had been rumen fistulated for previous experiments. The mordanted hay fiber was uniformly ground to 2 mm lengths to be representative of seed sizes. White sweetclover seed are oval and approximately 1.7 by 2.2 mm and narrowleaf hawksbeard seed are oblong and approximately 0.75 by 4 mm in size. During fecal collection, a subsample was removed for analysis of Cr content. Fecal subsamples were analyzed for Cr by inductive-coupled plasma mass spectroscropy to determine passage kinetics of 2 mm particles of Cr-mordanted fiber (Schwarm et al. 2008). ANOVA (SAS 2009) was used to detect differences ($P \leq 0.05$) between passage of germinable seed and Cr-mordanted fiber, differences among days after feeding, and their interaction. Mean separations of significant effects were evaluated with Fisher's Protected LSD test ($P \leq 0.05$).

Results and Discussion

No seedlings were observed in the silage tray, indicating there was no seed contamination from the silage diet. However, three plant species that were not intentionally fed to the moose were identified growing from the fecal material. These plant species were common lambsquarters (*Chenopodium album* L.), common chickweed [*Stellaria media* (L.) Vill.], and an unknown grass. There were 97 seedlings of these three species and both dicotyledonous species were common in the holding pens where the moose were acclimated to the silage before being placed in the digestion-balance stalls, and the dicotyledonous species had mature seed that the moose could consume.

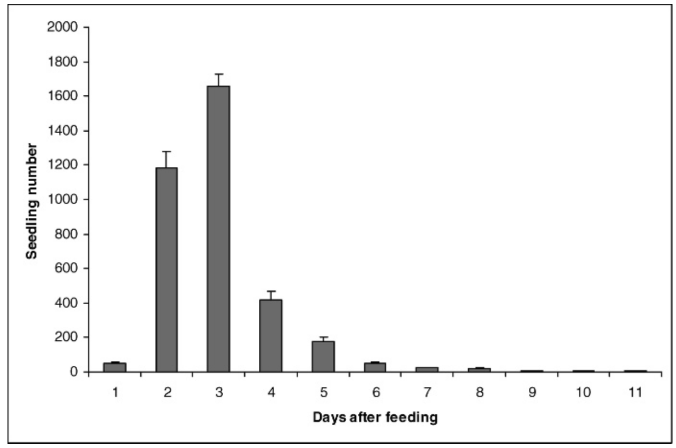

Figure 3. White sweetclover seedling totals observed in moose feces for four moose (combined). Bars represent standard error.

Of the 42,400 germinable narrowleaf hawksbeard seed that the moose were fed, only five seedlings were counted. Four of these seedlings were in a day-1 sample from one moose and one was from a day-3 sample from a second moose. Consequently, it appears that narrowleaf hawksbeard, which is morphologically adapted for wind dispersal, only rarely survived mastication and digestion in these moose.

Of the 22,000 germinable white sweetclover seed fed to the moose, 3,595 seedlings were counted in the fecal material, representing an 84% reduction due to mastication and/or digestion. There were no differences among the moose ($P = 0.06$) but number of seedlings per day varied significantly ($P < 0.0001$). Most of the seedlings were counted from feces produced 2 and 3 d after ingestion (Figure 3) and the timing of these peaks varied slightly among the moose (M1–M4) (Figure 4). The number of seedlings was reduced for all moose 5 d after feeding (DAF) (Figure 4a). Notably, no seedlings were counted from day 6 onward from M4, but the other moose still had seedlings 10 and 11 DAF. Individual differences in the moose, such as teeth condition, food intake, and time chewing cud all could impact seed survival.

Differences in passage kinetics did not change when seedling counts were presented as number per kg of dry weight feces (Figure 4b). Short-term (1 to 2 d) individual variations in passage have been observed with our moose in other experiments, underscoring the general protocol that requires ruminant digestion trials to last at least 10 d (William Collins, personal observation). These differences in seed passage are important from a perspective of how far a moose potentially could travel after ingestion before all the seed are defecated.

Overall passage kinetics of the 2-mm particles of Cr-mordanted fiber did not differ from seedling-producing seed ($P = 0.18$), but number of seedling producing seeds

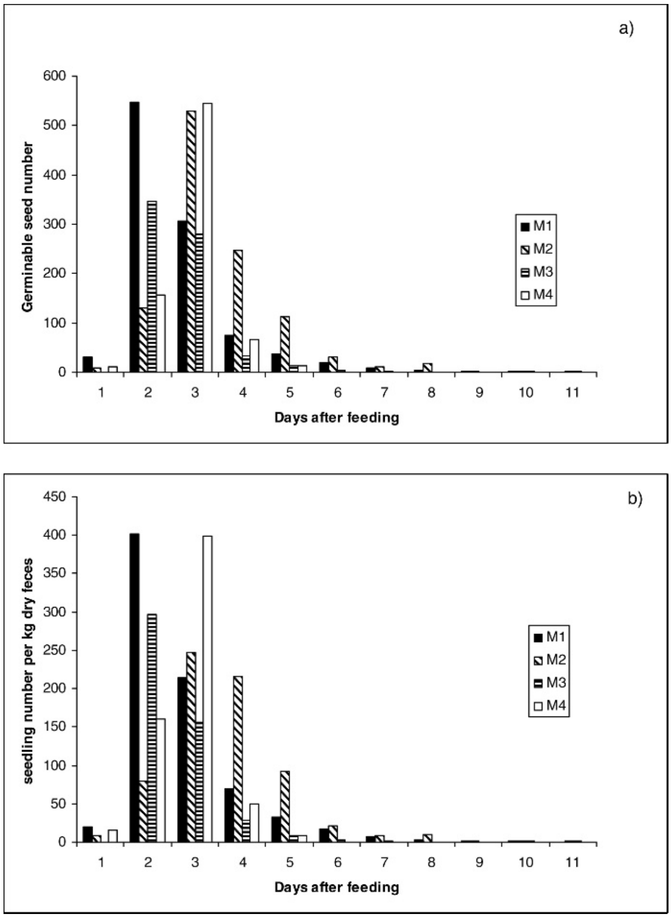

Figure 4. White sweetclover seedlings for (a) each of four moose (M1–M4) and (b) as a function of seedling number per kg dry feces.

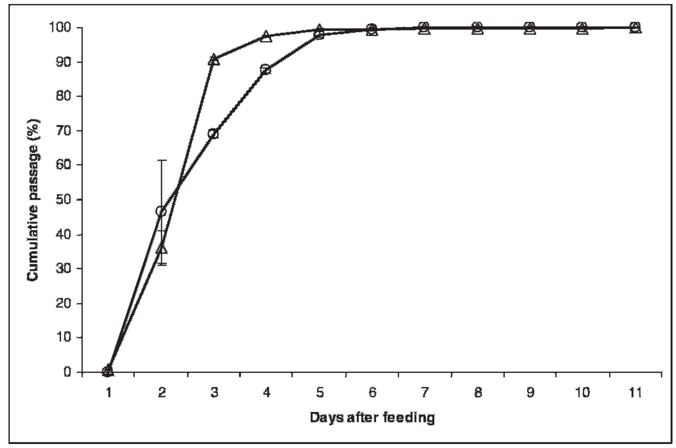

Figure 5. Comparison of cumulative excretion of white sweetclover seed (triangles) and 2 mm particles of Cr-mordanted hay (circles) in two moose. Bars represent standard error.

and Cr-mordanted fiber per day varied significantly ($P < 0.0001$). There was an interaction of these two effects ($P = 0.02$). At 3 and 4 DAF more of the seedling-producing seed passed from the moose than Cr-mordanted fiber (Figure 5). This seeming increase in the passage rate of the seedling-producing seed compared to the Cr-mordanted fiber could be a consequence of increased seed digestion and mastication with time in the moose as had been measured with leafy spurge ingested by sheep (Olson and Wallander 2002). Seedling-producing seeds most likely achieve a specific density that is more favorable for timely passage from the rumen than the specific density of the Cr-mordanted fiber. If total seed passage would have been measured directly, it is possible that passage of Cr-mordanted fiber would have been an accurate reflection of that of total seeds, which would imply that nonviable and nongerminable seeds are more similar in specific density to the mordants than germinable seeds. Measuring the specific density of seeds in relation to their viability possibly would allow prediction of their passage kinetics in comparison to well-researched markers.

In this study, 39% of the seedling-producing seed were inside the fecal pellets and did not emerge until the pellets were broken apart. In the field, moose fecal pellets often do not break down until after the next winter. This period can be longer in the dry interior (William Collins, personal observation).

Although germinable seed of white sweetclover declined 84% after passage through moose, there are probably enough germinable seed left to make endozoochorous dispersal a concern for land managers who are attempting to control white sweetclover. This method of dispersal is especially worrisome for those trying to keep this weed out of Alaska river systems because many roads, which tend to be infestation sources, occur along or nearby river channels. White sweetclover is a prolific seed producer with estimates of over 300,000 seed on a single plant grown without competition (Turkington et al. 1987). Currently, the Bureau of Land Management's (BLM) goal on their lands along the Dalton highway, which links Fairbanks to the north coast of Alaska, has been to keep roadsides within 30 m of a river crossing free of white sweetclover plants with hand weeding efforts (Ruth Gronquist, personal communication). Moose would be expected to move much farther in the 2 or 3 d after consumption when most of the seed will be deposited, so the BLM management program will need to be altered.

Potential distances for germinable seed dispersal by moose during late summer or early fall would be a function of retention time in the gut and distance traveled during that same period of time. Some moose are migratory, depending on the characteristics of their range (Sinclair 1983). If moose migrate from a summer range, they typically do not do so until snow depth increases, or travel to a rutting area becomes necessary. Because some moose might need to migrate to position themselves in optimal mating environments in early fall, potential exists for seeds ingested during that time to be transported considerable distances. Ballard et al. (1991)

measured migration distances of 16 to 93 km in a mountainous region of Alaska. Van Ballenberghe (1978) observed that, depending on conditions, moose required from 10 d to 6 wk to cover the same distance. Considering that ingested plant materials are generally retained in the moose gut for up to 11 d, it becomes evident that ingested seeds could be transported long distances. At the very least, it is likely that germinable seed could be transported the short distances typically separating rivers from the roads that cross or parallel them in Alaska.

Acknowledgments

The authors express their appreciation to Erin Carr, September Martin, Nick Cassara, and Jennifer Kapla for their technical assistance and Drs. Patrick Clark and Bret Taylor for comments to the manuscript. Partial funding was secured from the Alaska Department of Fish and Game, and the Swiss National Science Foundation. The University of Alaska Fairbanks Agricultural and Forestry Experiment Station, Matanuska Experiment Farm housed the moose and digestion balance stalls.

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Received May 12, 2009, and approved September 23, 2009.